IRIS Document

1988/09/01 — Document Date (MM/DD/YYYY)

Di(2-ethylhexyl) adipate — Chemical Name

10 — Sequence #

IRIS FILE TYPE

Circle One →

(IRIS Chemical File) Public Submission RfD/RfC & CRAVE Files

Subtype

Circle One

Decision files for chemicals listed in IRIS

Chemical nominations

CRAVE files prior to 1995

Toxicological Review

New Information

Non-decisional file reference and supplemental date prior to 1997

Peer review Record

Other

(Key/difficult to find materials)

Other

Other

Key Study - "Di(2-ethylhexyl) adipate: Teratogenicity Study in the Rat" CTL/P/2119

Description

ICI

Organization

Author

Scan Date

ICI CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

Sponsor: CEFIC
Sponsor Ref: -
CTL Ref: Y02259/003/003-4
CTL Study Nos: RR0372
Copy No: 26.

REPORT NO: CTL/P/2119

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

by

M C E Hodge

Gordon Steel.

Approved for Issue: G T Steel
Project Manager

Date of Issue:

85pp

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the above study.

M C E Hodge (Study Director) MCE Hodge 29 July 1988

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

The following contributed to this report in the capacities indicated:

J S Moore (Study Investigator) J.S. Moore 29.7.88

P B Banham (Analytical Chemist) P.B. Banham 1 Aug 1988

M R Greenwood (Statistician) M.R. Greenwood 1 Aug 1988

Reviewed by:
G A de S Wickramaratne (Senior Toxicologist) [signature] 2nd Aug '88

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

QUALITY ASSURANCE STATEMENT

In accordance with ICI policy for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Inspection/Audit	Date of QA Report
24 Sep 87	Protocol Audit	24 Sep 87
18 Sep 87	Inspections	18 Sep 87
24+25 Sep 87	Inspection	28 Sep 87
29+30 Sep 87	Inspection	30 Sep 87
7 Oct 87	Inspection	7 Oct 87
15 Jul 88	Draft Report Audit	18 Jul 88
5 Aug 88	Final Report Audit	5 Aug 88

In addition, facilities associated with this study were inspected according to Quality Assurance Standard Operating Procedures. So far as can be reasonably established, the methods described and the results given in this report accurately reflect the data produced during the study.

J R Pateman (Unit Head, CTL Quality Assurance Unit) ..... 12 Aug 88

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

CONTENTS

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

CONTENTS - continued

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

CONTENTS - continued

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

SUMMARY

Groups of 24 mated female Alpk:APfSD rats were fed diets containing 0, 300, 1800 or 12000ppm di(2-ethylhexyl)adipate (DEHA) from days 1-22 of gestation. A dietary method of administration was selected as being most like that of the probable human exposure. The achieved concentration was within 8% of target and the doses received by the test groups were approximately 28, 170 or 1080mg DEHA/kg/day.

The day of mating was designated day 1 of gestation. On day 22, the females were killed and their uteri examined for live foetuses and intra-uterine deaths. The foetuses were weighed, examined for external abnormalities, sexed, eviscerated (the viscera were examined for abnormalities) and stained for subsequent skeletal examination for defects and degree of ossification (including a *manus* and *pes* scoring).

Administration of 12000ppm DEHA resulted in a small but statistically significant reduction in maternal bodyweight gain when compared to the control group, particularly at the start of gestation. There was also a small but statistically significant reduction in food consumption at this dose level from days 2-18 inclusive of gestation. These effects indicate that 12000ppm was a suitable dose level at which to evaluate the effects of DEHA on development *in utero*. There was no evidence of maternal toxicity at 300 or 1800ppm DEHA.

There was no effect at any dose on foetal weight, litter weight, gravid uterus weight, numbers of intra-uterine deaths or numbers of external abnormalities. At 12000ppm DEHA, there was a minimal increase in pre-implantation loss with an associated decrease in litter size.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

SUMMARY - continued

Six major abnormalities (in five foetuses) were seen in the treated groups and eight in the control group (of which seven consisted of multiple minor skull defects in one litter). There was no evidence that the type or distribution of these abnormalities was related to treatment with DEHA.

The incidence of minor external and visceral defects was unaffected by treatment although two visceral variants were increased at the top two dose levels; kinked ureter being increased in the 1800 and 12000ppm groups and slightly dilated ureter being increased in the 12000ppm group. Overall, minor skeletal defects were increased in a dose-related manner at 1800 and 12000ppm DEHA, while skeletal variants (as a percentage of foetuses affected) were increased at the top dose only. These findings indicate slightly poorer ossification at the 1800 and 12000ppm DEHA dose levels and both they and the increased incidence of variants of the ureter are considered to be the result of slight foetotoxicity.

It is therefore concluded that DEHA administered to rats in the diet throughout gestation caused slight maternal toxicity at the top dose level (12000ppm) and slight but dose-related foetotoxicity at 1800 and 12000ppm as shown by reduced ossification and minor changes in the ureter. A dietary level of 300ppm was shown to be a clear no-effect level and there was no evidence at any dose level that DEHA is teratogenic to the rat.

1. INTRODUCTION

Di(2-ethylhexyl)adipate (DEHA) is a plasticiser for polyvinyl chloride particularly for low temperature applications. The purpose of this study was to investigate the effects of DEHA on the embryonic and foetal development of the rat when administered in the diet during pregnancy.

The rat is one of the species generally recommended for assessment of teratogenicity and the Alpk:APfSD (Wistar-derived) strain was used because of the substantial background data within this Laboratory relating to studies of this type. The oral route was chosen for administration of DEHA and dietary administration was used as this was considered to be most akin to the method of human exposure since DEHA is an indirect food contaminant.

The dose levels selected for this study were based on information obtained from the literature with the top dose representing the limit dose (1000mg/kg/day) recommended by the Organisation for Economic Cooperation and Development (OECD) guideline number 414. The bottom dose was related to likely human exposure. The maximum human intake has been estimated by MAFF (UK) 1986 to be 16mg/day and this was calculated to be 0.25mg/kg/day for a 60-70kg human. A factor of 100 was then used to provide an appropriate margin of safety which thus gave a dose of 25mg/kg/day in rats for the present study. The middle dose was spaced between these two doses using approximately a sixfold factor. The dose levels were then calculated as ppm in the diet (for a 300g rat eating 25g food per day). The rats were dosed on Days 1-22 inclusive of gestation, Day 1 being the day that mating was confirmed by a sperm-positive vaginal smear.

The in life phase of the study was conducted from 15 September to 16 October 1987. Original data obtained in this study are retained in the Archives at the ICI Central Toxicology Laboratory (CTL) and copies of the report are lodged with the CTL Report Centre.

2. MATERIALS AND METHODS

2.1 Test Substance

DEHA, was supplied by ICI France, Department Baleycourt, as a colourless liquid. The batch used was identified by the CTL reference numbers Y02259/003/003-4. The purity was analysed to be ,99.2% w/w and a correction was made when calculating the quantities of DEHA to be incorporated into the diets. Analytical details are shown in Appendix A.

2.2 Diet Preparation

All diets were based on CT1 diet supplied by Special Diets Services Ltd, Witham, Essex, UK. The constituents of CT1 are shown in Appendix B. The experimental diets were prepared in 30kg batches from premixes as described in Appendix C and dispensed into glass feeding jars. Two batches of diet were prepared at each level.

2.3 Diet Sampling and Analysis

A sample was taken from each diet prepared. Samples were taken from the diet feeding jars and analysed as detailed in Appendix D. Chemical stability of DEHA in CT1 diet was determined at 300 and 12000ppm. Additional stability data from a concurrent study are presented in Appendix E.

Homogeneity of DEHA was also examined in a concurrent study (Tinston 1988) and found to be satisfactory.

2.4 Animals and Husbandry

Wistar-derived, virgin female rats of the Alpk:APfSD strain (from the Specific Pathogen Free (SPF) colony, maintained at the Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK)

were paired overnight at the Breeding Unit with unrelated males of the same strain. On the following morning, vaginal smears from these females were examined for the presence of sperm.

The day when spermatozoa were detected was designated Day 1 of gestation and on this same day, successfully mated females were delivered to the experimental unit at CTL.

A total of 96 mated females was supplied over a two week period. On arrival, the rats were within the weight range 218-278g and were approximately 12 weeks of age. Twelve female rats were supplied on each of eight days.

For the duration of the study, each rat was individually housed in rat racks supplied by All Type Tools Ltd, Woolwich, London, UK. The cages had solid stainless steel sides and the floor, back and front were constructed of 14SWG stainless steel mesh. The internal measurements were 34.0 x 37.5 x 20.3cm with a floor area of 1275cm^2. The cages were suspended over collecting trays lined with absorbent paper. On the front of each cage was a card identifying the animal by individual number, dose group and study. Tap water via an automatic watering system and food were available ad libitum.

The temperature of the animal room was within the range of 19-24°C (as recorded daily by a maximum and minimum thermometer) with a mean of 22°C. Relative humidity was within a recorded range of 44-70% (as assessed by daily readings from a hygrometer) and mean of 54%. There were at least 12 air changes per hour. The artificial lighting was controlled by a time switch and provided alternate periods of 12 hours light and 12 hours darkness throughout the study.

2.5 Experimental Design

The study consisted of four groups each containing 24 rats as shown below:

TABLE 1
EXPERIMENTAL DESIGN

Group	Dose Level of DEHA (ppm)	Animal Numbers
1	0 (control)	1 - 24
2	300	25 - 48
3	1800	49 - 72
4	12000	73 - 96

The study was divided into 24 replicates (randomised blocks) with each replicate containing one rat from each dosage group. Cages within the replicates were assigned to one of the four groups using computer-generated random number permutations. The individual animal numbers were then assigned sequentially within the relevant groups to give the rack plan shown in Appendix F. On arrival (Day 1 of gestation) each rat was allocated to a cage (and therefore a treatment group) randomly within the replicate and individually identified by ear punching with the number assigned to it from the experimental design. Replicates were filled sequentially with three replicates added to the study on each of the eight days on which rats were received.

2.6 Dosing

All animals received their appropriate experimental diet from Day 1 of gestation until termination on Day 22.

2.7 Experimental Observations

2.7.1 Clinical Observations: All animals were checked on arrival to ensure that they were physically normal externally. They were subsequently observed daily for any changes in behaviour or clinical condition and these were recorded.

2.7.2 Bodyweights: The bodyweight of each animal was recorded daily on Days 1 to 22 inclusive of gestation.

2.7.3 Food Consumption: The amount of food consumed by each animal was measured daily by giving a weighed quantity of food contained in a glass jar on one day and calculating the amount consumed from the residue on the next.

2.7.4 Terminal Investigations: On Day 22 of gestation all the animals were killed by over exposure to halothane BP (FLUOTHANE, ICI Pharmaceuticals, Macclesfield, Cheshire, UK) vapour. A post mortem was performed and all animals were examined macroscopically.

The intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries and uterus were then examined and the following data recorded:-

Number of corpora lutea in each ovary.
Number and position of implantations subdivided into:

(a) live foetuses.
(b) early intra-uterine deaths.
(c) late intra-uterine deaths.

Intra-uterine deaths were classified as follows: Early intra-uterine deaths showed decidual or placental tissue only. Late intra-uterine deaths showed embryonic or foetal tissue in addition to placental tissue.

The implantations were assigned letters of the alphabet to identify their position *in utero* starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. In addition, each foetus was weighed and individually identified within the litter by means of a cardboard tag.

After weighing, the foetuses were killed with an intra-cardiac injection of pentobarbitone sodium solution, 200mg/ml, (EUTHATAL, May and Baker Ltd, Dagenham, Essex, UK).

2.7.5 Assessment of Teratogenicity: Each foetus was examined for external abnormalities and for cleft palate. All foetuses were then examined internally for visceral abnormalities under magnification, sexed, eviscerated and fixed in methanol. The head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. (The brains of one litter, female 72, 1800ppm, inadvertently were not examined.) The carcasses were then returned to methanol for subsequent processing and staining with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed. The individual bones of the *manus* and *pes* were assessed and the result converted to a four point scale as detailed in Appendix G.

Abnormalities were classified as major (rare or possibly lethal or both) or minor (deviations from normal that are not uncommon at external, visceral or skeletal examination) defects. Variations were also recorded and classified as minor defects or variants depending on the historical frequency of occurrence in rats of this strain.

2.8 Statistical Analysis

Data from one non-pregnant animal (from the 12000ppm group) and from one animal with total resorptions (from the 300ppm group) were excluded from the statistical analyses (and the Figures).

The following data were considered by analysis of variance:

(i) Maternal bodyweight gain.

(ii) Maternal food consumption.

(iii) The numbers of implantations and live foetuses per female.

(iv) Percentage pre-implantation loss and percentage post-implantation loss (calculated on an individual litter basis), defined as:

% Pre-implantation loss =

$$\frac{\text{No. of corpora lutea - No. of implantations}}{\text{No. of corpora lutea}} \times 100$$

% Post- implantation loss =

$$\frac{\text{No. of implantations - No. of live foetuses}}{\text{No. of implantations}} \times 100$$

The percentage pre-implantation loss and post-implantation loss were transformed before analysis using the double arcsine transformation of Freeman and Tukey (1950). The analyses of variances were weighted by the denominator in the proportion.

(v) The percentage of implantations which were early intra-uterine deaths (calculated on an individual litter basis). The percentage was transformed before analysis using the double arcsine transformation and the analysis of variance was weighted by the number of implantations in each litter.

(vi) Gravid uterus weight, litter weight and mean foetal weight (calculated on an individual litter basis). The analysis of mean foetal weight was weighted by the number of foetuses in each litter.

(vii) Mean manus and pes score per foetus (calculated on an individual litter basis). The analyses were weighted by the number of foetuses in each litter.

(viii) The percentage of foetuses with minor external/visceral defects only, external/visceral variants and minor skeletal defects only (calculated on an individual litter basis). The percentages were transformed before analysis using the double arcsine transformation and the analyses were weighted by the number of foetuses examined in each litter.

The analyses of variance allowed for the replicate structure of the study design and were carried out using the GLM procedure in SAS (1985). Unbiased estimates of the treatment group means were provided by the least square means (LSMEANS option in SAS). Individual treatment group means were compared with the control group mean using Student's t-test based on the error mean square in the analysis.

The following parameters were analysed by Fisher's Exact Test, comparing each treated group with the control group:

(i) The proportion of females with pre-implantation loss.

(ii) The proportion of females with post-implantation loss.

(iii) The proportion of females with early intra-uterine deaths.

(iv) The proportion of females with late intra-uterine deaths.

(v) The proportion of foetuses which were male.

(vi) The proportion of foetuses with major or minor (only) external/visceral defects, major or minor (only) skeletal defects, external/visceral variants, skeletal variants and specific findings. The proportion of foetuses with specific findings was also analysed on a litter basis.

All statistical tests were one-sided with the following exceptions which were two-sided: maternal bodyweight gain, maternal food consumption and the proportion of male foetuses.

3. RESULTS

3.1 Diet Analysis (Tables 2 and 3)

Dietary concentrations of DEHA were within 8% of target values (Table 2).

Chemical stability was determined on diets prepared for this study at 300 and 12000ppm DEHA (Table 3). Satisfactory chemical stability was observed at 300ppm up to at least 32 days. This interval is in excess of the maximum period of use of the first batch of diet (21 days from preparation). At 12000ppm an interim analysis after 14 days showed a significant fall in concentration but with a return to a higher mean concentration at 32 days. Chemical stability was determined at the same concentration levels on three occasions in a concurrent study (Tinston 1988). These data shown in Appendix E indicate satisfactory chemical stability at both concentrations for up to 34 days. It is therefore believed that the low interim value seen in this study at 12000ppm after 14 days is a spurious result and that chemical stability of DEHA in diet is satisfactory.

3.2 Clinical Observations (Table 4)

All rats survived to scheduled termination.

The incidence of clinical findings was low and they were of a type commonly seen in rats of this age and strain. They were considered not to be related to DEHA administration.

3.3 Maternal Bodyweight Gain (Table 5, Figure 1)

Administration of 12000ppm DEHA was associated with a small but statistically significant reduction in bodyweight gain compared with the control group which was most marked at the start of the feeding period.

There were no adverse effects on maternal weight gain at 300 or 1800ppm DEHA and bodyweight gain was very similar in these dose groups to that of the control group.

3.4 Maternal Food Consumption (Table 6, Figure 2) and Dose Received (Figure 3)

Maternal food consumption was statistically significantly reduced in the 12000ppm group from Days 2-18 inclusive of pregnancy. There were no adverse effects on food consumption in the 300 or 1800ppm DEHA groups.

The dose received is shown graphically in Figure 3 and can be seen to be approximately 28, 170 or 1080mg/kg/day in the 300, 1800 or 12000ppm DEHA groups respectively (based on nominal dietary levels).

It should be noted that food consumption in all groups and dose received in the test groups were lower for the last day, reflecting a decrease in intake caused by removing animals for autopsy..

3.5 Maternal Macroscopic Findings Post Mortem (Table 7)

Few of the animals showed macroscopic changes. The changes were of a type and incidence commonly seen in the Alpk:APfSD rat and were considered not to be related to treatment with DEHA.

3.6 Litter Data (Table 8)

The only difference between the test and control groups was a small increase in the pre-implantation loss in the 12000ppm DEHA group. This was associated with a small reduction in the number of implantations

and live foetuses. There was also a minimal increase in post implantation loss but the incidence was within control incidences for recent studies (Appendix H). None of these differences was statistically significant and there were no effects at 300 or 1800ppm DEHA.

3.7 Foetal Abnormalities (Tables 9-12)

3.7.1 Major Defects (Tables 9 and 10): Major defects were seen in 13 foetuses. Seven of these (all from female number 7 in the control group) had multiple minor defects, particulary of the skull and were therefore classified as having major defects. Excluding these seven foetuses, the incidence of major defects was 1, 2, 1, 2 in the 0, 300, 1800 and 12000ppm DEHA groups respectively. Foetus 13C (control group) had an absent adrenal, kidney and ureter. In the 300ppm DEHA group, one foetus (43A) had cysts attached to the liver and foetus 47E had a small right kidney. Neither of these abnormalities have been seen in recent studies. Foetuses 60B (1800ppm DEHA) and 95C (12000ppm DEHA) had a major defect of the vertebral column and ribs while 95C also had an umbilical hernia. Foetus 80F (12000ppm DEHA) had <u>situs inversus totalis</u>.

The low incidence of these defects indicates that they were spontaneous and unrelated to DEHA administration.

3.7.2 Minor Defects (Tables 9 and 11, Figure 4): The incidence of foetuses with minor external and/or visceral defects was low and not increased by treatment with DEHA.

Overall, minor skeletal defects were increased in a dose-related manner at both 1800ppm DEHA and 12000ppm DEHA. The only defect to show a clear dose response was partially ossified parietals of the skull. Not ossified centra of the 3rd-7th cervical vertebrae were also higher in these two dose groups.

Bipartite 5th sternebra was higher in all DEHA treated groups, although only at 12000ppm were the values clearly above recent controls (Figure 4).

The following minor defects had increased incidences in the 12000ppm DEHA dose group only; partially ossified occipitals of the skull, not ossified ventral tubercle of the cervical vertebrae, bipartite centra of the 11th and 12th thoracic vertebra, slightly misaligned 3rd and 4th sternebrae, and thickened mid point of the 10th rib.

All or most of the recorded incidences of the following skull defects were due to the affected foetuses of control female 7; partially ossified frontals, partially ossified mandible, partially ossified maxilla, partially ossified nasals, anterior and posterior fontanelle widened slighty. Incidences in the control group of kinked ribs (5th to 12th) and ribs with thickened mid point (5th to 11th) were also mainly foetuses of female 7.

3.7.3 Variants (Tables 9 and 11, Figures 5 and 6): Only two external and visceral variants were recorded (slightly dilated ureter and kinked ureter) which combined and individually show a slight increase with increasing dose of DEHA. The background control incidences of these two defects (Figures 5 and 6) are decreasing slowly with time and suggest that the values seen in the 12000ppm group (both variants) and the 1800ppm group (kinked ureter) fall outside the range expected.

Skeletal variants were increased in the 12000ppm DEHA dose group only. Specific defects which were increased at this dose level were: not ossified calcaneum, partially ossified 5th sternebra, transverse processes of the 7th cervical vertebra partially ossified (also higher at 1800ppm DEHA in a dose related manner). The higher incidence of not ossified odontoid was considered not to be related to treatment with DEHA due to the general lack of coherent dose response. The higher incidences of fully or partially ossified transverse processes of the 4th lumbar vertebra indicate a slight increase in ossification for this one parameter in all three treatment groups although again there was no clear dose response and therefore this was unlikely to be treatment-related.

3.7.4 Manus and Pes Assessment (Table 12): The mean manus and pes scores were analysed with and without female 7 whose foetuses (G, H, I, J, K, L, M - described earlier, 3.7.1) mainly had values of 4 representing two-thirds of all such scores recorded. Pes scores were slightly higher in the 12000ppm DEHA dose group.

4. DISCUSSION

There was no evidence of disease or infection amongst the animals. Environmental control was satisfactory. Analysis of the diets showed that the concentrations of DEHA were within acceptable limits and that the homogeneity [which was determined in a concurrent study (Tinston 1988)] and chemical stability of DEHA in diet were satisfactory.

In the 12000ppm DEHA group, there was a small reduction in maternal bodyweight gain compared to the control group which was most marked at the start of the feeding period. Food consumption was reduced throughout most of gestation but not on Day 1 suggesting that the cause was toxicity and not palatability. Bodyweight gain and food consumption in the 300 and 1800pm groups were not affected by treatment. There were no treatment-related clinical observations or macroscopic findings at post mortem examination in any group.

The slight maternal toxicity observed at 12000ppm DEHA demonstrates that a maximum tolerated dose was achieved while the dose received in mg/kg/day was within 10% of the limit level recommended by the OECD. For either of these reasons, the study is suitable for the evaluation of the developmental effects of DEHA.

There was no effect at any dose on foetal weight, litter weight, gravid uterus weight, numbers of intra-uterine deaths or numbers of external abnormalities. At 12000ppm DEHA, there was a minimal increase in pre-implantation loss with an associated decrease in litter size. However, these differences were not statistically significant and they were too small to be of toxicological significance.

Six major abnormalities (in five foetuses) were seen in the treated groups and eight in the control group (of which seven consisted of multiple minor skull defects in one litter).

There was no evidence that the type or distribution of these abnormalities was related to treatment.

The incidence of minor external and visceral defects was unaffected by treatment although two visceral variants were increased at the top two dose levels; kinked ureter being increased in the 1800 and 12000ppm groups and slightly dilated ureter being increased in the 12000ppm group. Overall, minor skeletal defects were increased in a dose-related manner at 1800 and 12000ppm DEHA, while skeletal variants and pes score were increased at the top dose only. These findings indicate slightly poorer ossification at the 1800 and 12000ppm dose levels. The reduced ossification and increase in the incidence of visceral variants are considered to be the result of slight foetotoxicity. There was no treatment-related effect on skeletal or visceral variants at 300ppm DEHA.

5. CONCLUSION

There was no evidence that DEHA is teratogenic to the rat at any of the dose levels tested (up to the OECD limit level of 1000mg/kg/day).

Administration of 12000ppm DEHA resulted in slight maternal toxicity and slight foetotoxicity.

At 1800ppm DEHA, there was no evidence of maternal toxicity although minimal foetotoxicity was observed.

A dietary level of 300ppm DEHA was a clear no-effect level for embryonic development.

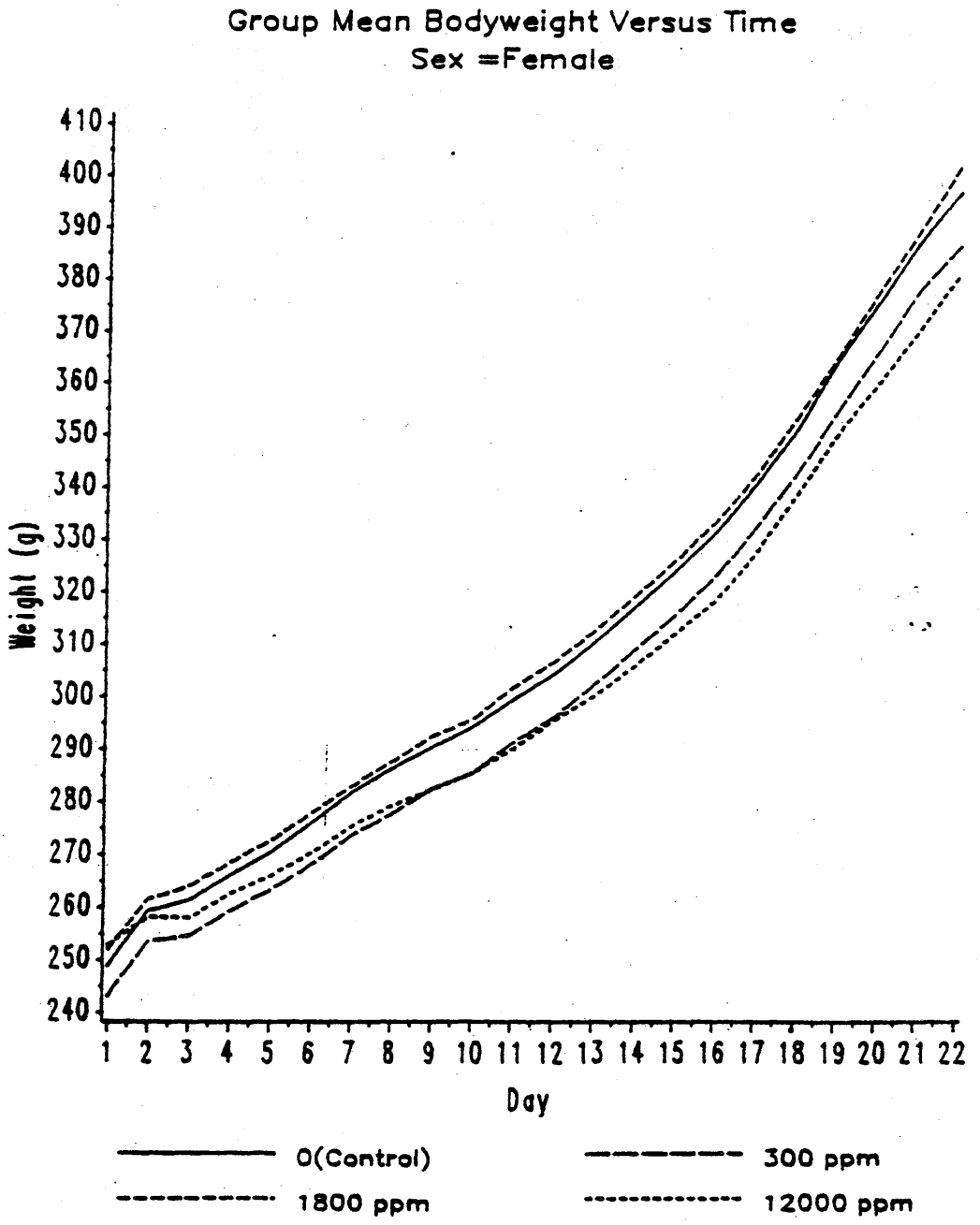
6. REFERENCES

Freeman M F and Tukey J W (1950). Transformation related to the angular and the square root. Annals of Maths Stats 21, 607.

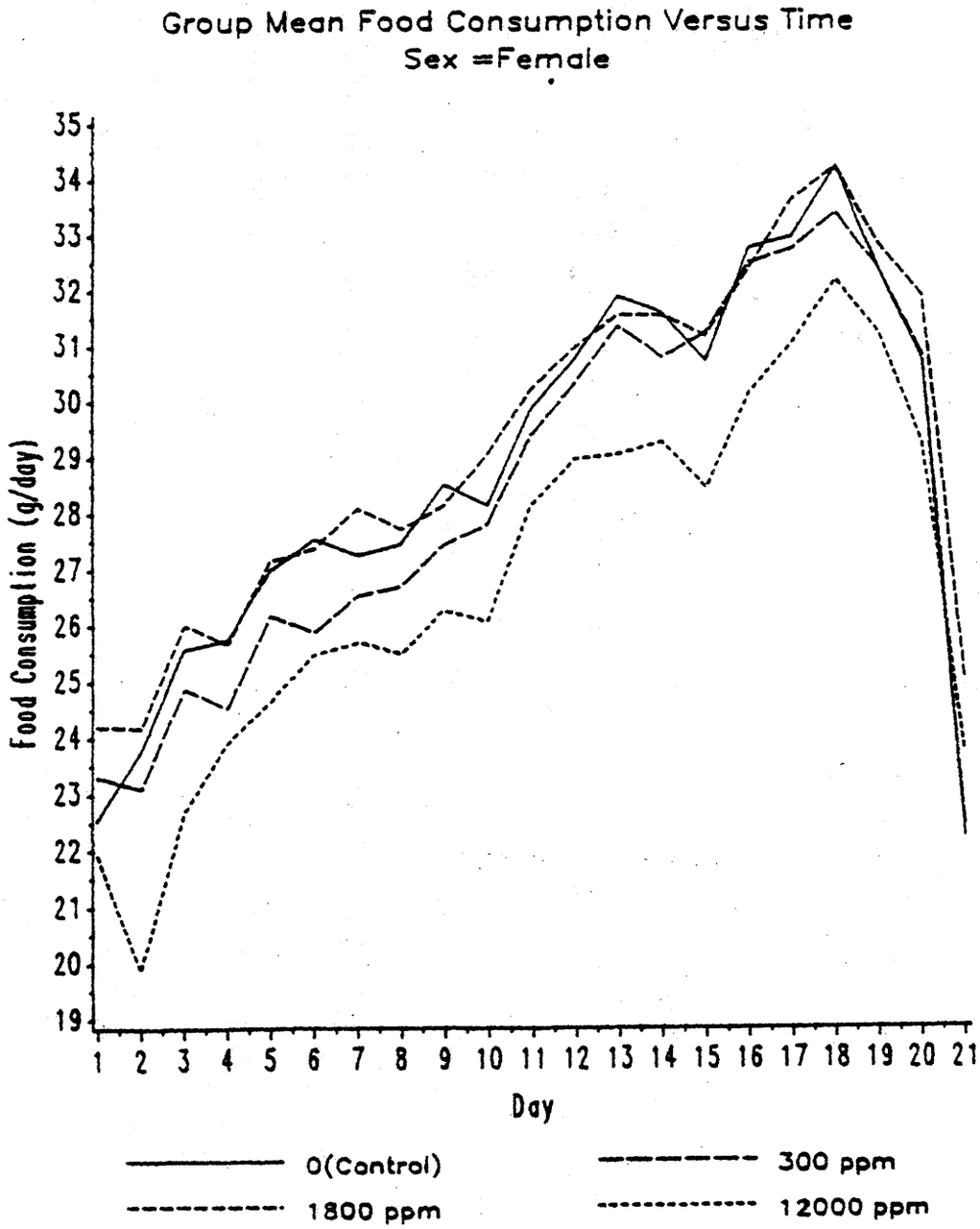
SAS Institute Inc. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC: SAS Institute Inc, 1985.

Tinston D J (1988). Di-(2-ethylhexyl)adipate (DEHA): Fertility Study in Rats. ICI Central Toxicology Laboratory. Report No CTL/P/2229.

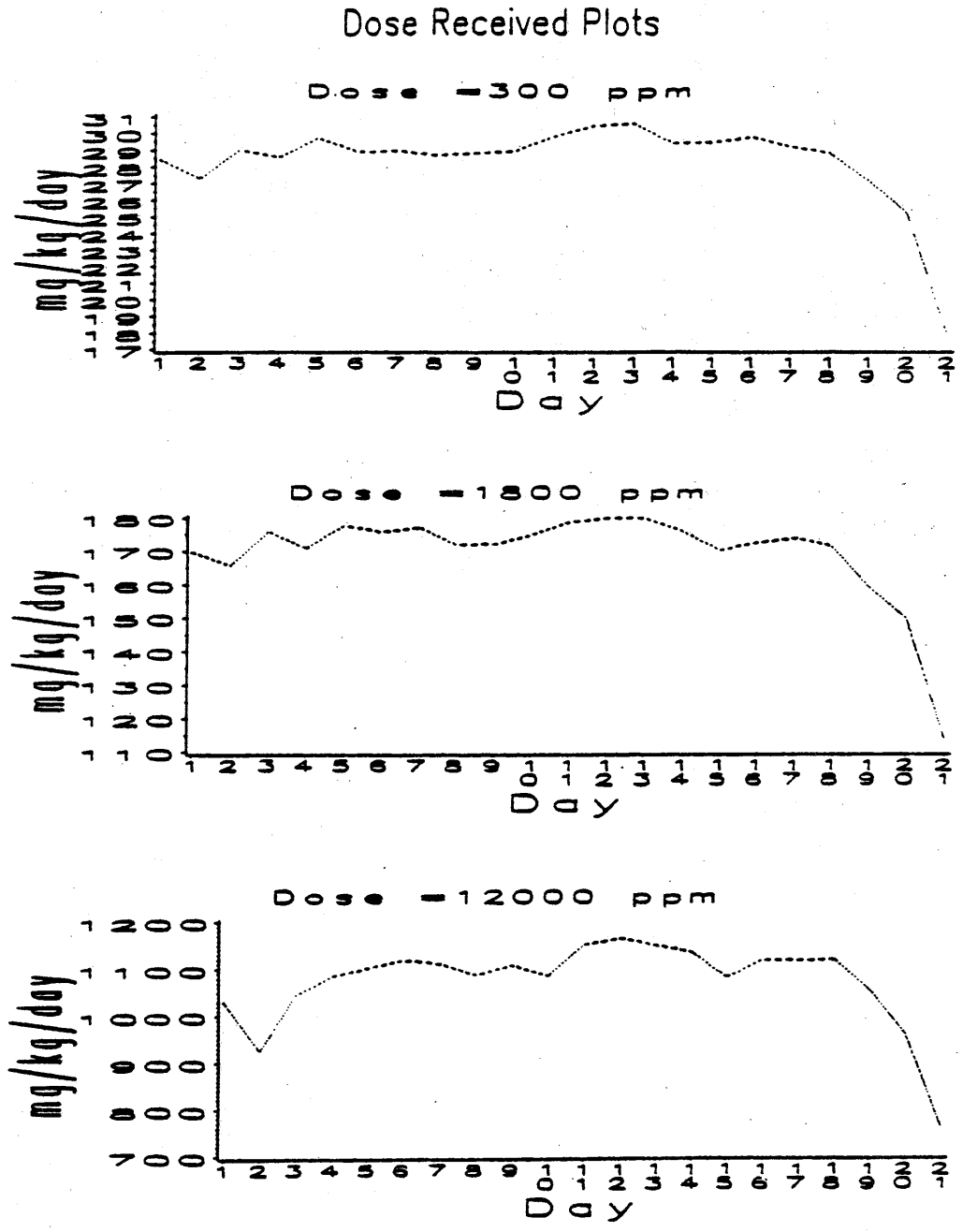
DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT
FIGURE 1
MATERNAL BODYWEIGHTS



DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT
FIGURE 2
MATERNAL FOOD CONSUMPTION



DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT
FIGURE 3
DOSE RECEIVED



DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

GLOSSARY FOR FIGURES 4, 5 AND 6

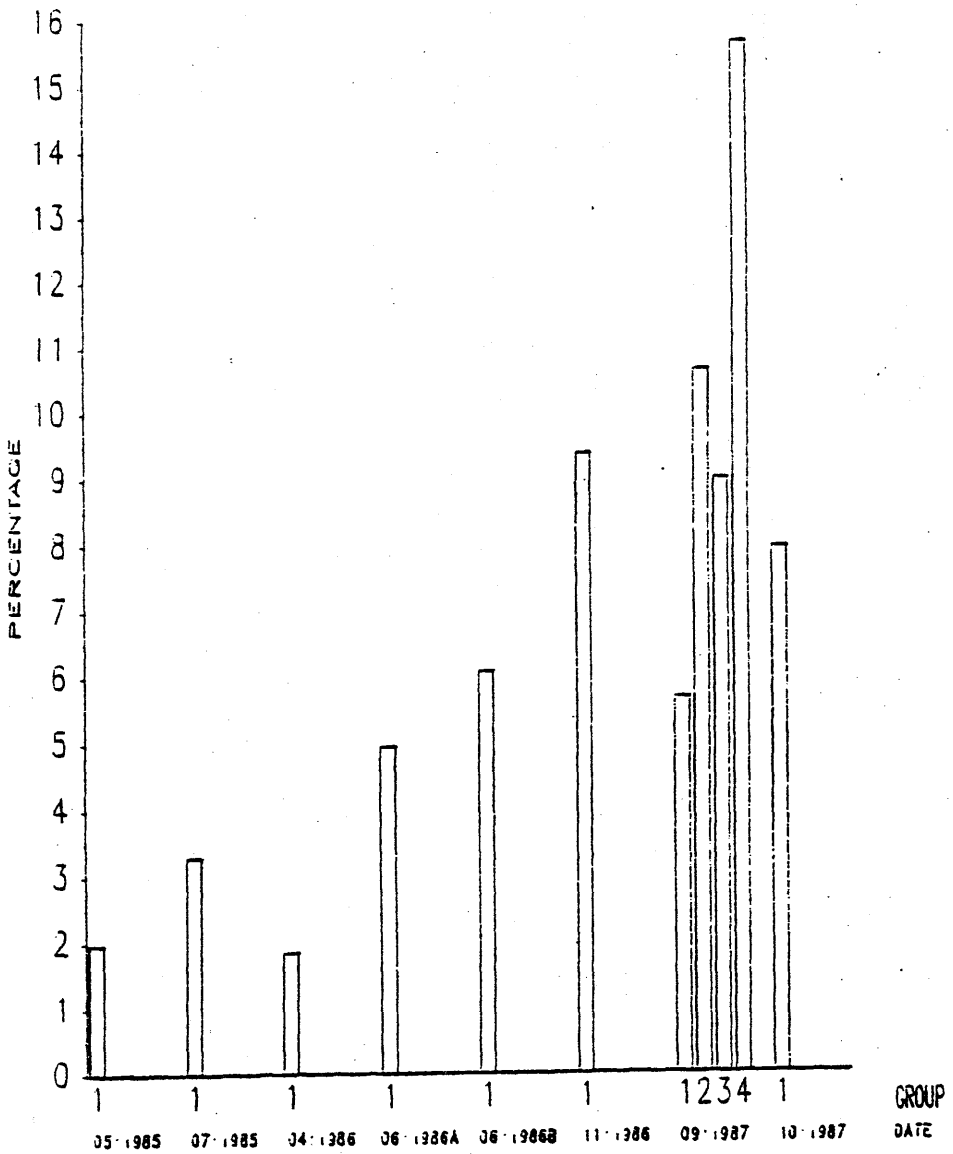
DATE refers to the month/year in which the bulk of the live phase of the studies shown was undertaken.

Data from all groups (ie, 1, 2, 3 and 4) in the present study are shown while data from other studies are restricted to the control group (1).

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

FIGURE 4

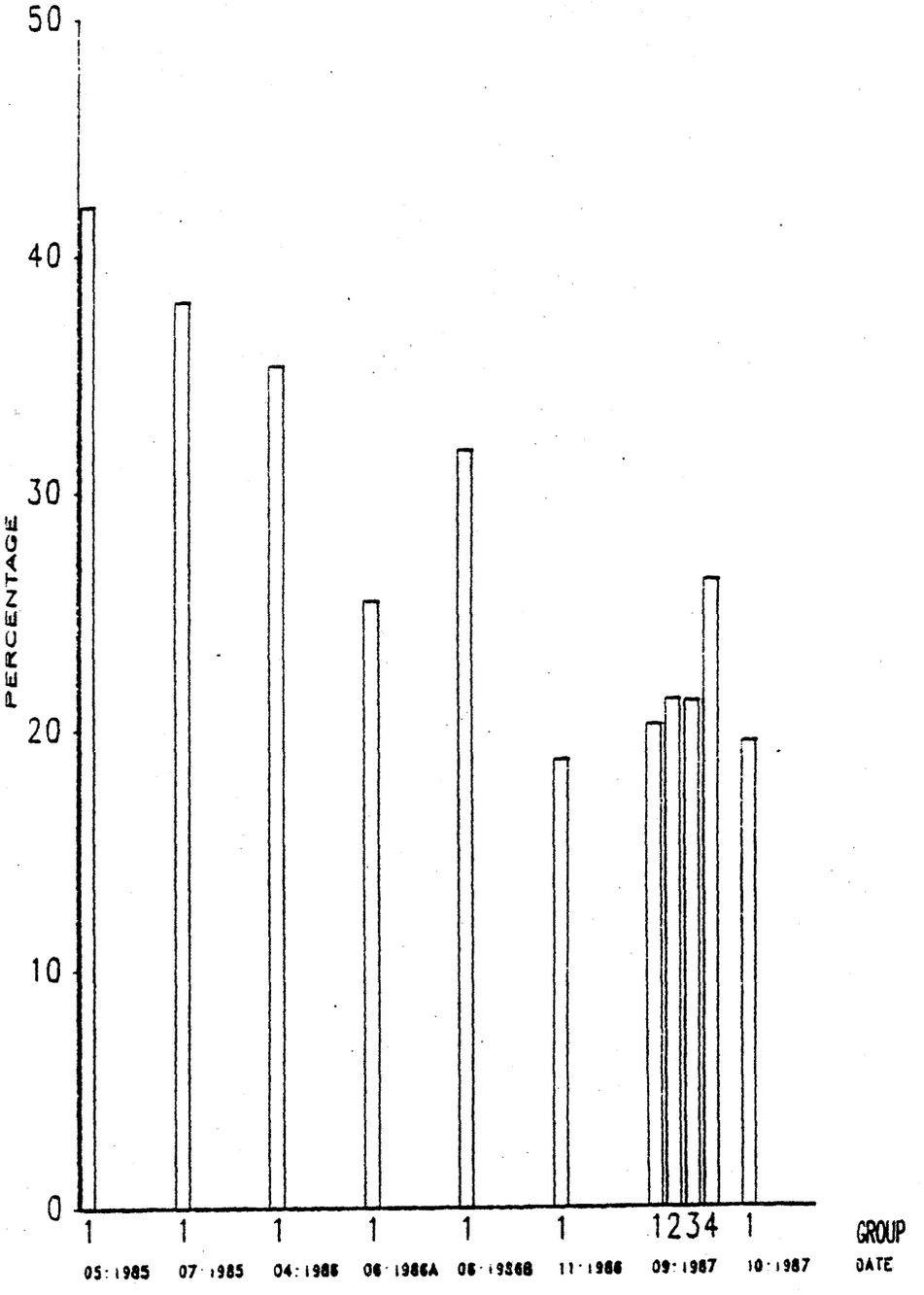
HISTORICAL CONTROL PLOTS
PERCENTAGE OF FOETUSES WITH BIPARTITE 5th STERNEBRAE



DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

FIGURE 5

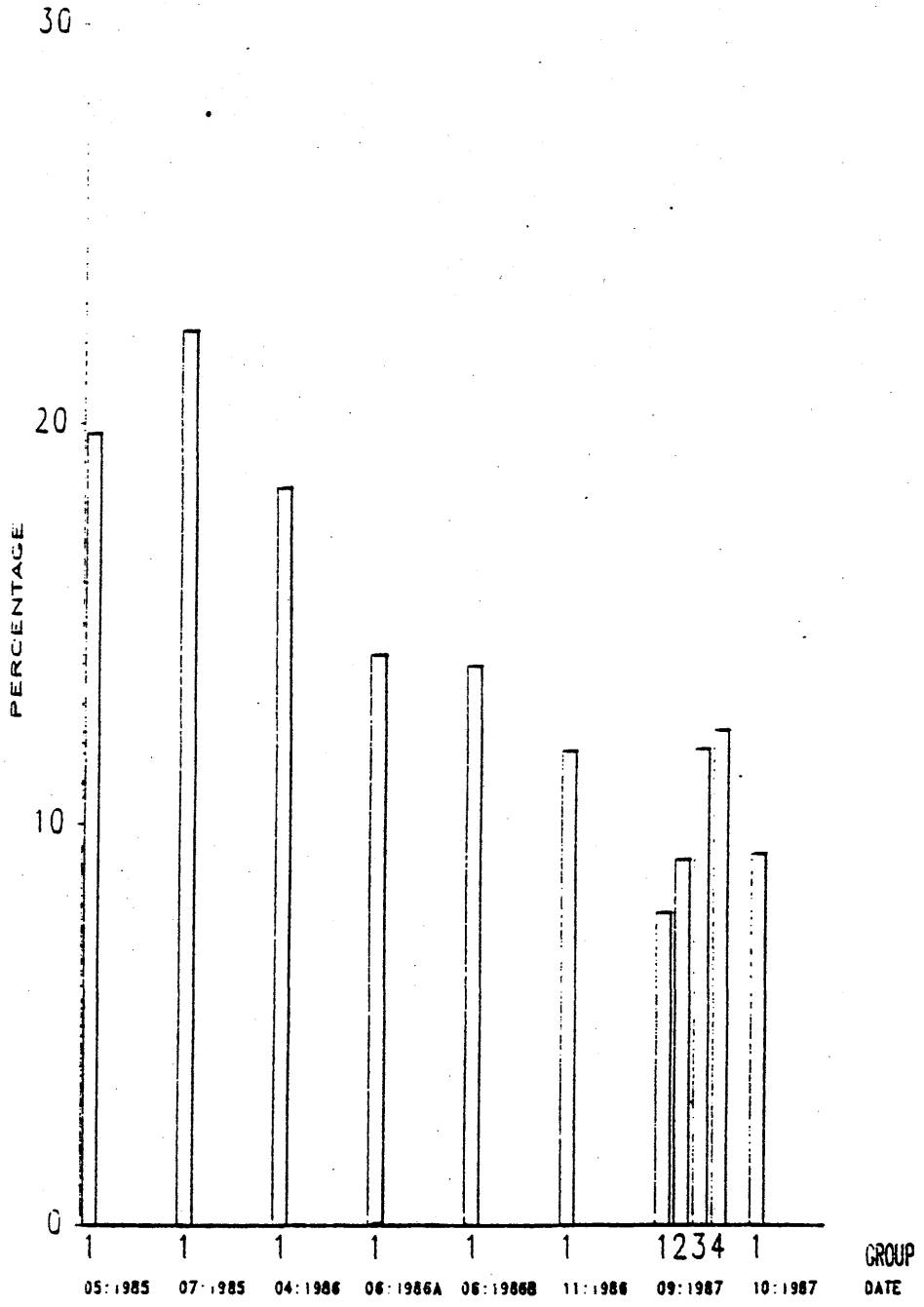
HISTORICAL CONTROL PLOTS
PERCENTAGE OF FOETUSES WITH SLIGHTLY DILATED URETERS



DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

FIGURE 6

HISTORICAL CONTROL PLOTS
PERCENTAGE OF FOETUSES WITH KINKED URETERS



DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 2

ACHIEVED CONCENTRATIONS OF DEHA IN DIET

Preparation Date	Nominal Concn (ppm w/w)	Analysed Concn (ppm w/w)	Mean Analysed Concn (ppm w/w)	% of Nominal
11 Sep 87	0 (Control)	ND		
	300	287, 278	283	94.3
	1800	1712, 1674	1693	94.1
	12000	12340, 12110	12230	101.9
30 Sep 87	0 (Control)	ND		
	300	300, 294	297	99.0
	1800	1904, 1791	1848	102.7
	12000	11270, 10860	11070	92.3

ND = not detected, detection limit 10ppm.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 3

CHEMICAL STABILITY OF DEHA IN DIET

Preparation Date	Nominal Concn (ppm w/w)	Analysis Date	Analysis Interval (days)	Analysed Concn (ppm w/w)	Mean Concn (ppm w/w)	% of Initial Value
11 Sep 87	300	11 Sep 87	0	287 278	283	100.0
		25 Sep 87	14	275 269	272	96.1
		13 Oct 87	32	262 255	259	91.5
	12000	11 Sep 87	0	12340 12110	12230	100.0
		25 Sep 87	14	9982 10330	10160	83.1
		13 Oct 87	32	10710 11470	11090	90.7

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 4

SUMMARY OF CLINICAL OBSERVATIONS (ALL ANIMALS)

SEX: FEMALE	0 PPM		300 PPM		1800 PPM		12000 PPM	
COAT STAINED 1/MORE AREAS								
NO. OF OBS.	1		5		14			
NO. OF ANIMALS	1		1		1			
DAYS FROM - TO	8	8	9	13	8	21		
DRY SORES 1 OR MORE AREAS								
NO. OF OBS.							3	
NO. OF ANIMALS							1	
DAYS FROM - TO							4	6
EXOPHTHALMUS								
NO. OF OBS.					17			
NO. OF ANIMALS					1			
DAYS FROM - TO					6	22		
HAIR LOSS VENTRALLY								
NO. OF OBS.			17		5			
NO. OF ANIMALS			1		1			
DAYS FROM - TO			6	22	18	22		
HAIR LOSS 1 OR MORE AREAS								
NO. OF OBS.	21		26		48		1	
NO. OF ANIMALS	1		2		4		1	
DAYS FROM - TO	1	22	13	22	9	22	22	22
CHROMODACRYORRHEA LEFT								
NO. OF OBS.			17					
NO. OF ANIMALS			2					
DAYS FROM - TO			1	18				
SCABS 1 OR MORE AREAS								
NO. OF OBS.	11		3					
NO. OF ANIMALS	1		1					
DAYS FROM - TO	7	17	1	3				
TAIL DAMAGED								
NO. OF OBS.					22		21	
NO. OF ANIMALS					1		1	
DAYS FROM - TO					1	22	2	22

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

GLOSSARY FOR STATISTICAL TABLES 5, 6, 8, 9, 11 and 12

Means for all tables are based on the number of females with live foetuses in utero at termination (Day 22) unless otherwise indicated in parentheses.

Means are least square means where confidence limits are presented.

The approximate 95% confidence limit for each group mean is based on the error mean square in the analysis of variance and is calculated as the average 95% confidence limit for each individual group mean.

Key to results of statistical test:

* Statistically significant difference from control at the 5% level.

** Statistically significant difference from control at the 1% level.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 5

MATERNAL BODYWEIGHT GAIN (g)

INTERGROUP COMPARISON OF MATERNAL BODYWEIGHT GAIN (g) - excluding Total Resorptions

Period (Days)	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
Initial Weight (Day 1)	248.9	243.1	252.1	252.9	-
1-2	10.6	10.1	9.5	5.5**	±2.8
1-3	12.6	11.5	11.9	5.2**	±2.0
1-4	17.2	16.2	16.4	9.8**	±2.2
1-5	21.6	20.2	20.6	13.0**	±2.4
1-6	27.1	24.9	25.9	17.4**	±2.7
1-7	32.9	30.4	31.0	22.4**	±2.8
1-8	37.5	34.8	35.7	26.4**	±2.9
1-9	41.6	39.4	40.5	29.6**	±3.2
1-10	45.5	42.5	43.8	32.9**	±3.4
1-11	50.6	48.3	49.8	37.3**	±3.5
1-12	55.6	53.2	54.8	42.9**	±3.7

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 5 - continued

MATERNAL BODYWEIGHT GAIN (g)

INTERGROUP COMPARISON OF MATERNAL BODYWEIGHT GAIN (g) - excluding Total Resorptions

Period (Days)	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
1-13	61.6	59.3	60.6	47.4**	±3.9
1-14	68.4	66.0	67.3	53.4**	±4.3
1-15	75.2	72.4	74.0	59.4**	±4.7
1-16	82.5	80.0	81.3	65.7**	±4.8
1-17	91.8	89.3	90.5	74.9**	±5.2
1-18	102.4	99.8	101.6	86.3**	±5.9
1-19	115.7	111.7	113.3	98.1**	±6.7
1-20	126.6	122.8	125.6	107.9**	±7.2
1-21	138.3	134.7	137.3	118.1**	±7.7
1-22	148.0	143.5	149.8	129.3**	±8.2
Final Weight (Day 22)	396.9	386.6	401.9	382.8	±10.7
Number of females with live foetuses in utero at termination.	24	23	24	23	

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 6

MATERNAL FOOD CONSUMPTION (g/animal/day)

INTERGROUP COMPARISON OF MATERNAL FOOD CONSUMPTION (g/day) - excluding Total Resorptions

Period (Days)	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
1	22.5	23.4	24.2	22.0	±1.5
2	23.8	23.2	24.2	19.9**	±1.0
3	25.6	24.8	26.0	22.6**	±1.0
4	25.8	24.8	25.7	23.8**	±1.0
5	27.0	26.2	27.2	25.0**	±1.0
6	27.5	25.9*	27.4	25.6**	±1.0
7	27.3	26.6	28.1	25.8*	±1.1
8	27.5	26.7	27.7	25.6**	±1.0
9	28.5	27.4	28.1	26.3**	±1.0
10	28.1	27.9	29.0	26.0**	±1.0
11	29.9	29.4	30.2	28.2*	±1.1

TABLE 6 - continued

MATERNAL FOOD CONSUMPTION (g/animal/day)

INTERGROUP COMPARISON OF MATERNAL FOOD CONSUMPTION (g/day) - excluding Total Resorptions

Period (Days)	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
12	30.8	30.4	31.0	29.0*	±1.1
13	31.9	31.3	31.5	29.1**	±1.1
14	31.6	30.7	31.5	29.3**	±1.2
15	30.7	31.4	31.2	28.4**	±1.0
16	32.8	32.5	32.4	30.2**	±1.2
17	33.0	32.7	33.6	31.2*	±1.2
18	34.2	33.5	34.2	32.3*	±1.2
19	32.3	32.6	32.8	31.3	±1.0
20	30.7	31.0	31.9	29.4	±1.3
21	22.2	22.6	25.0	24.0	±2.5
Total (1-21)	603.5	594.9	612.8	565.0**	±16.6
Number of females with live foetuses in utero at termination.	24	23	24	23	

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 7

MATERNAL MACROSOCOPIC FINDINGS POST MORTEM (DAY 22)

Description of Findings	Dose Level of DEHA (ppm)			
	0	300	1800	12000
Number of females examined at termination	24	24	24	24
Number of females showing: No abnormalities detected	17	17	17	14
Liver: accentuated reticular pattern	0	2	3	4
Spleen: numerous white cysts on surface	0	0	0	1
Kidney: slight pelvic dilation	1	4	1	2
moderate pelvic dilatation	4	0	2	3
extreme pelvic dilatation	0	0	1	0
fatty mass on surface	1	0	0	0
fatty cyst on surface	0	0	1	1
discoloured (light/pale brown)			1	
enlarged	2	0	1	2
pitted	0	0	1	0
Stomach: slightly distended with gas	0	1	0	0
empty	0	1	0	0
Rectum: contains gas	0	0	0	1
slightly distended with gas	1	0	0	0
moderately distended with gas	1	1	0	0
empty	0	1	0	0
Ovary: cystic bursa	1	1	0	2
area of haemorrhaging	0	1	0	0
Abdominal cavity: clotted dark red/black material	0	1	0	0
Pelvic cavity: small white granules	0	0	1	0
clear fluid	0	0	1	1

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 8

LITTER DATA

INTERGROUP COMPARISON OF LITTER DATA

	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
Number of females mated	24	24	24	24	-
Number of females with live foetuses in utero at termination	24	23	24	23	-
Mean no. of corpora lutea	14.2	13.4	13.7	13.9	-
Pre-implantation loss					
Percentage	13.5	12.0	11.6	19.1	-
Mean transformed value	0.345	0.358	0.323	0.430	±0.093
Prop. of females affected	14/24	17/23	14/24	17/23	
Mean no. of implantations	12.3	11.7	12.1	11.3	±1.3
Post-implantation loss					
Percentage	4.1	3.0	4.1	5.8	-
Mean transformed value	0.230	0.205	0.216	0.256	±0.059
Prop. of females affected	9/24	6/23	8/24	8/23	
Mean no. of live foetuses	11.8	11.3	11.6	10.7	±1.4

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 8 - continued

LITTER DATA

INTERGROUP COMPARISON OF LITTER DATA

	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
Intra-uterine deaths					
Number early	12	8	12	13	-
Percentage	4.1	3.0	4.1	5.0	-
Mean transformed value	0.231	0.206	0.217	0.246	±0.057
Prop. of females affected	9/24	6/23	8/24	8/23	
Number late	0	0	0	2	-
Percentage	0.0	0.0	0.0	0.8	-
Prop. of females affected	0/24	0/23	0/24	2/23	

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 8 - continued

LITTER DATA

INTERGROUP COMPARISON OF LITTER DATA

	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
Total no. of live foetuses	282	263	278	243	-
Prop. of male foetuses	138/282	132/263	131/278	121/243	
Percentage	48.9	50.2	47.1	49.8	-
Mean gravid uterus weight (g)	83.7	81.4	84.9	78.0	±8.8
Mean litter weight (g)	59.0	57.1	59.3	53.6	±6.6
Mean foetal weight (g)	5.04	5.03	5.14	5.02	±0.12

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 9

FOETAL DEFECTS AND VARIANTS

INTERGROUP COMPARISON OF FOETAL DEFECTS AND VARIANTS

	Dietary Concentration of DEH Adipate (ppm) 0(Control)	300	1800	12000	Approx 95% Conf Limit
No. of litters examined	24	23	24	23	-
External and visceral defects					
No. of foetuses examined	282	263	278	243	-
No. of foetuses showing major defects	1	2	0	2	-
Percentage	0.4	0.8	0.0	0.8	-
No. of foetuses showing minor defects only	7	8	9	3	-
Percentage	2.5	3.0	3.2	1.2	-
Mean transformed value	0.183	0.209	0.200	0.182	±0.049
Variants					
No. of foetuses showing variants	69	69	81	78*	-
Percentage	24.5	26.2	29.1	32.1	-
Mean transformed value	0.486	0.506	0.556	0.597	±0.130

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 9 - continued

FOETAL DEFECTS AND VARIANTS

INTERGROUP COMPARISON OF FOETAL DEFECTS AND VARIANTS

	Dietary Concentration of DEH Adipate (ppm)				Approx 95%
	0(Control)	300	1800	12000	Conf Limit
Skeletal defects					
No. of foetuses examined	282	263	278	243	-
No. of foetuses showing major defects	7	0	1	1	-
Percentage	2.5	0.0	0.4	0.4	-
No. of foetuses showing minor defects only	70	78	97**	120**	-
Percentage	24.8	29.7	34.9	49.4	-
Mean transformed value	0.518	0.586	0.628*	0.776**	±0.078
Variants					
No. of foetuses showing variants	270	257	268	243**	-
Percentage	95.7	97.7	96.4	100.0	-

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 10

SUMMARY OF THE TYPE AND INCIDENCE OF MAJOR DEFECTS

	Dose Level of DEHA (ppm)			
	0	300	1800	12000
External/Visceral				
Situs Inversus Totalis	0	0	0	1(80F)
Left adrenal, kidney and ureter absent	1(13C)	0	0	0
Cysts attached to liver	0	1(43A)	0	0
Small right kidney	0	1(47E)	0	0
Umbilical hernia	0	0	0	1(95C)
Skeletal				
Skull:				
Multiple minor defects	7 (7G) (7H) (7I) (7J) (7K) (7L) (7M)	0	0	0
Vertebral Column (Thoracic)/Rib:				
3rd arch (left) not ossified 6th and 7th arches (left) fused 2nd, 6th and 8th centra misshapen 3rd and 7th (left) hemicentra not ossified 4th centrum misshapen slightly 2nd through to 13th arches misaligned 3rd and 7th ribs (left) not ossified	0	0	0	1(95C)

Foetus identity is given in parentheses.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 10 - continued

SUMMARY OF THE TYPE AND INCIDENCE OF MAJOR DEFECTS

	Dose Level of DEHA (ppm)			
	0	300	1800	12000
Vertebral Column/Rib:				
3rd and 4th cervical arch (right) fused One (unidentified) left arch not ossified 5th and 6th thoracic arches (left) fused 3rd cervical through to 11th thoracic arches misaligned 6th thoracic hemicentrum (left) not ossified 5th thoracic centrum misshapen 7th and 8th thoracic centra bipartite and displaced 5th and 6th ribs (left): slight fusion 1st rib (right) partially ossified	0	0	1(60B)	0

Foetus identity is given in parentheses.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

GLOSSARY FOR TABLE 11

For each defect the total number and percentage of foetuses affected is given in the first line and the number and percentage of litters affected is given on the second line.

CLASS denotes classification, ie major, minor or variant.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
EXTERNAL/VISCERAL DEFECTS									
EXTERNAL/VISCERAL									
NO ABNORMALITIES DETECTED		210	(74.5)	189	(71.9)	197	(70.9)	164	(67.5)
		24	(100)	22	(95.7)	24	(100)	23	(100)
TORSO									
SITUS INVERSUS TOTALIS	MAJ	0		0		0		1	(0.4)
		0		0		0		1	(4.3)
SUBCUTANEOUS HAEMORRHAGE	MIN	1	(0.4)	1	(0.4)	0		1	(0.4)
		1	(4.2)	1	(4.3)	0		1	(4.3)
INOMINATE ARTERY									
ABSENT-RIGHT CAROTID,SUBCLAVIAN ARTERIES SEPARATE	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
ABDOMEN									
UMBILICAL HERNIA	MAJ	0		0		0		1	(0.4)
		0		0		0		1	(4.3)
LIVER									
CYST(S) ATTACHED	MAJ	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
PALE	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
ADRENALS									
ABSENT - UNILATERAL	MAJ	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
KIDNEY									
ABSENT - UNILATERAL	MAJ	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
PELVIS DILATED - UNILATERAL - SLIGHTLY	MIN	2	(0.7)	1	(0.4)	2	(0.7)	0	
		1	(4.2)	1	(4.3)	1	(4.2)	0	
SMALL - UNILATERAL	MAJ	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
✓ URETER									
DILATED - UNILATERAL - MODERATELY	MIN	6	(2.1)	6	(2.3)	9	(3.2)	2	(0.8)
		2	(8.3)	5	(21.7)	6	(25.0)	2	(8.7)
DILATED - UNILATERAL - SLIGHTLY	VAR	57	(20.2)	56	(21.3)	59	(21.2)	64	(26.3)
		12	(50.0)	16	(69.6)	17	(70.8)	19*	(82.6)
✓ KINKED - UNILATERAL	VAR	22	(7.8)	24	(9.1)	33	(11.9)	30	(12.3)
		12	(50.0)	14	(60.9)	13	(54.2)	16	(69.6)
ABSENT - UNILATERAL	MAJ	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
SKELETAL DEFECTS									
SKELETAL									
MULTIPLE MINOR DEFECTS, FOETUS	MIN	7	(2.5)	0		0		0	
CLASSIFIED MAJOR	MAJ	1	(4.2)	0		0		0	
NO ABNORMALITIES DETECTED		8	(2.8)	6	(2.3)	8	(2.9)	0	
		7	(29.2)	5	(21.7)	7	(29.2)	0	
SKULL									
FRONTALS - PARTIALLY OSSIFIED	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
INTERPARIETAL - PARTIALLY OSSIFIED	MIN	17	(6.0)	12	(4.6)	26	(9.4)	16	(6.6)
		10	(41.7)	6	(26.1)	11	(45.8)	7	(30.4)
MANDIBLE - UNILATERAL - PARTIALLY OSSIFIED	MIN	7	(2.5)	0		0		0	
		1	(4.2)	0		0		0	
MAXILLA - UNILATERAL - PARTIALLY OSSIFIED	MIN	7	(2.5)	0		0		0	
		1	(4.2)	0		0		0	
NASALS - PARTIALLY OSSIFIED	MIN	6	(2.1)	0		0		0	
		1	(4.2)	0		0		0	
OCCIPITAL - PARTIALLY OSSIFIED	MIN	1	(0.4)	1	(0.4)	2	(0.7)	7*	(2.9)
		1	(4.2)	1	(4.3)	2	(8.3)	3	(13.0)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
SKULL									
PARIETALS - UNILATERAL - PARTIALLY OSSIFIED	MIN	11	(3.9)	8	(3.0)	22*	(7.9)	24**	(9.9)
		7	(29.2)	3	(13.0)	8	(33.3)	10	(43.5)
SKULL:SUTURAL BONES									
BETWEEN INTERPARIETAL AND PARIETALS	MIN	1	(0.4)	1	(0.4)	2	(0.7)	0	
		1	(4.2)	1	(4.3)	2	(8.3)	0	
SKULL:FONTANELLE									
ANTERIOR - WIDENED SLIGHTLY	MIN	7	(2.5)	0		0		0	
		1	(4.2)	0		0		0	
POSTERIOR - WIDENED SLIGHTLY	MIN	7	(2.5)	0		2	(0.7)	1	(0.4)
		2	(8.3)	0		2	(8.3)	1	(4.3)
ODONTOID									
NOT OSSIFIED	VAR	65	(23.0)	81*	(30.8)	83*	(29.9)	55	(22.6)
		21	(87.5)	21	(91.3)	21	(87.5)	19	(82.6)
CERVICAL VERTEBRAE									
ARCH PARTIALLY OSSIFIED,3RD - UNILATERAL	MIN	0		0		1	(0.4)	2	(0.8)
		0		0		1	(4.2)	2	(8.7)
ARCH PARTIALLY OSSIFIED,4TH - UNILATERAL	MIN	0		0		1	(0.4)	2	(0.8)
		0		0		1	(4.2)	2	(8.7)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		0 ppm		300 ppm		1800 ppm		12000 ppm	
	CLASS	INCIDENCE BY FOETUS/LITTER							
		NO.	%	NO.	%	NO.	%	NO.	%
CERVICAL VERTEBRAE									
ARCH PARTIALLY OSSIFIED,5TH - UNILATERAL	MIN	0		0		1	(0.4)	2	(0.8)
		0		0		1	(4.2)	2	(8.7)
ARCH PARTIALLY OSSIFIED,6TH - UNILATERAL	MIN	0		0		1	(0.4)	2	(0.8)
		0		0		1	(4.2)	2	(8.7)
ARCH PARTIALLY OSSIFIED,7TH - UNILATERAL	MIN	0		0		0		1	(0.4)
		0		0		0		1	(4.3)
NOT OSSIFIED, VENTRAL TUBERCLE	MIN	11	(3.9)	6	(2.3)	8	(2.9)	26**	(10.7)
		7	(29.2)	5	(21.7)	7	(29.2)	12	(52.2)
CENTRUM NOT OSSIFIED,2ND	VAR	135	(47.9)	135	(51.3)	130	(46.8)	101	(41.6)
		24	(100)	23	(100)	24	(100)	22	(95.7)
CENTRUM NOT OSSIFIED,3RD	MIN	22	(7.8)	21	(8.0)	29	(10.4)	28	(11.5)
		14	(58.3)	12	(52.2)	11	(45.8)	11	(47.8)
CENTRUM NOT OSSIFIED,4TH	MIN	8	(2.8)	11	(4.2)	11	(4.0)	13	(5.3)
		6	(25.0)	10	(43.5)	7	(29.2)	7	(30.4)
CENTRUM NOT OSSIFIED,5TH	MIN	3	(1.1)	4	(1.5)	5	(1.8)	11*	(4.5)
		3	(12.5)	4	(17.4)	4	(16.7)	5	(21.7)
CENTRUM NOT OSSIFIED,6TH	MIN	1	(0.4)	0		2	(0.7)	5	(2.1)
		1	(4.2)	0		2	(8.3)	5	(21.7)
CENTRUM NOT OSSIFIED,7TH	MIN	0		0		2	(0.7)	3	(1.2)
		0		0		2	(8.3)	2	(8.7)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
CERVICAL VERTEBRAE									
TRANSVERSE PROCESS FULLY OSSIFIED,7TH - UNILATERAL	MIN	5	(1.8)	6	(2.3)	5	(1.8)	4	(1.6)
		5	(20.8)	4	(17.4)	3	(12.5)	3	(13.0)
TRANSVERSE PROCESS PARTIALLY OSSIFIED,7TH - UNILATERAL	VAR	65	(23.0)	59	(22.4)	88*	(31.7)	94**	(38.7)
		19	(79.2)	19	(82.6)	22	(91.7)	22	(95.7)
RIB(S) ON 7TH - UNILATERAL	MIN	0		3	(1.1)	2	(0.7)	3	(1.2)
		0		3	(13.0)	2	(8.3)	3	(13.0)
THORACIC VERTEBRAE									
ARCH PARTIALLY OSSIFIED,1ST - UNILATERAL	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
CENTRUM BIPARTITE,2ND	MIN	0		0		0		1	(0.4)
		0		0		0		1	(4.3)
CENTRUM BIPARTITE,4TH	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
CENTRUM BIPARTITE,8TH	MIN	0		0		0		1	(0.4)
		0		0		0		1	(4.3)
CENTRUM BIPARTITE,11TH	MIN	1	(0.4)	1	(0.4)	1	(0.4)	5	(2.1)
		1	(4.2)	1	(4.3)	1	(4.2)	5	(21.7)
CENTRUM BIPARTITE,12TH	MIN	0		0		1	(0.4)	5*	(2.1)
		0		0		1	(4.2)	5*	(21.7)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		0 ppm		300 ppm		1800 ppm		12000 ppm	
	CLASS	INCIDENCE BY FOETUS/LITTER							
		NO.	%	NO.	%	NO.	%	NO.	%
THORACIC VERTEBRAE									
CENTRUM BIPARTITE,13TH	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
CENTRUM PARTIALLY OSSIFIED,1ST	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
CENTRUM PARTIALLY OSSIFIED,3RD	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
CENTRUM PARTIALLY OSSIFIED,4TH	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
CENTRUM PARTIALLY OSSIFIED,8TH	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
CENTRUM PARTIALLY OSSIFIED,11TH	MIN	1	(0.4)	0		0		1	(0.4)
		1	(4.2)	0		0		1	(4.3)
CENTRUM PARTIALLY OSSIFIED,12TH	MIN	0		0		1	(0.4)	1	(0.4)
		0		0		1	(4.2)	1	(4.3)
CENTRUM PARTIALLY OSSIFIED,13TH	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
HEMICENTRUM PARTIALLY OSSIFIED,1ST - UNILATERAL	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
HEMICENTRUM PARTIALLY OSSIFIED,2ND - UNILATERAL	MIN	0		0		0		1	(0.4)
		0		0		0		1	(4.3)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
THORACIC VERTEBRAE									
HEMICENTRUM PARTIALLY OSSIFIED,3RD - UNILATERAL	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
HEMICENTRUM PARTIALLY OSSIFIED,4TH - UNILATERAL	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
LUMBAR VERTEBRAE									
CENTRUM BIPARTITE,1ST	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
CENTRUM BIPARTITE,3RD	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
HEMICENTRUM PARTIALLY OSSIFIED, 3RD - UNILATERAL	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
TRANSVERSE PROCESSES									
OF 3RD LUMBAR PARTIALLY OSSIFIED - UNILATERAL	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
OF 4TH LUMBAR FULLY OSSIFIED - UNILATERAL	VAR	59	(20.9)	62	(23.6)	77*	(27.7)	73*	(30.0)
		17	(70.8)	18	(78.3)	18	(75.0)	20	(87.0)
OF 4TH LUMBAR PARTIALLY OSSIFIED - UNILATERAL	VAR	159	(56.4)	179**	(68.1)	160	(57.6)	158*	(65.0)
		24	(100)	23	(100)	24	(100)	23	(100)
OF 5TH LUMBAR PARTIALLY OSSIFIED - UNILATERAL	MIN	1	(0.4)	0		1	(0.4)	2	(0.8)
		1	(4.2)	0		1	(4.2)	2	(8.7)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
TRANSVERSE PROCESSES									
OF 6TH LUMBAR PARTIALLY OSSIFIED	MIN	1	(0.4)	0		0		1	(0.4)
- UNILATERAL		1	(4.2)	0		0		1	(4.3)
VERTEBRAL COLUMN									
MAJOR DEFECT	MAJ	0		0		1	(0.4)	1	(0.4)
		0		0		1	(4.2)	1	(4.3)
27 PRE-SACRAL VERTEBRAE	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
STERNEBRAE									
BIPARTITE,1ST	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
BIPARTITE,2ND	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
BIPARTITE,4TH	MIN	0		0		1	(0.4)	1	(0.4)
		0		0		1	(4.2)	1	(4.3)
BIPARTITE,5TH	MIN	16	(5.7)	28*	(10.6)	25	(9.0)	38**	(15.6)
		6	(25.0)	14*	(60.9)	15**	(62.5)	15**	(65.2)
BIPARTITE,6TH	MIN	0		1	(0.4)	1	(0.4)	0	
		0		1	(4.3)	1	(4.2)	0	
MISALIGNED EXTREMELY,4TH	MIN	0		0		0		1	(0.4)
		0		0		0		1	(4.3)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		0 ppm		300 ppm		1800 ppm		12000 ppm	
	CLASS	INCIDENCE BY FOETUS/LITTER							
		NO.	%	NO.	%	NO.	%	NO.	%
STERNEBRAE									
MISALIGNED EXTREMELY,5TH	MIN	0		0		0		1	(0.4)
		0		0		0		1	(4.3)
MISSHAPEN,1ST	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
MISSHAPEN 2ND	MIN	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
NOT OSSIFIED,5TH	MIN	1	(0.4)	2	(0.8)	5	(1.8)	3	(1.2)
		1	(4.2)	2	(8.7)	4	(16.7)	3	(13.0)
NOT OSSIFIED,6TH	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
PARTIALLY OSSIFIED,1ST	MIN	0		0		1	(0.4)	1	(0.4)
		0		0		1	(4.2)	1	(4.3)
PARTIALLY OSSIFIED,2ND	MIN	1	(0.4)	0		0		2	(0.8)
		1	(4.2)	0		0		2	(8.7)
PARTIALLY OSSIFIED,4TH	MIN	0		0		0		1	(0.4)
		0		0		0		1	(4.3)
PARTIALLY OSSIFIED,5TH	VAR	95	(33.7)	83	(31.6)	89	(32.0)	119**	(49.0)
		19	(79.2)	20	(87.0)	21	(87.5)	22	(95.7)
PARTIALLY OSSIFIED,6TH	MIN	1	(0.4)	1	(0.4)	1	(0.4)	3	(1.2)
		1	(4.2)	1	(4.3)	1	(4.2)	3	(13.0)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		0 ppm		300 ppm		1800 ppm		12000 ppm	
	CLASS	INCIDENCE BY FOETUS/LITTER							
		NO.	%	NO.	%	NO.	%	NO.	%
STERNEBRAE									
MISALIGNED SLIGHTLY,2ND	MIN	1	(0.4)	1	(0.4)	0		0	
		1	(4.2)	1	(4.3)	0		0	
MISALIGNED SLIGHTLY,3RD	MIN	0		0		1	(0.4)	3	(1.2)
		0		0		1	(4.2)	3	(13.0)
MISALIGNED SLIGHTLY,4TH	MIN	1	(0.4)	0		1	(0.4)	3	(1.2)
		1	(4.2)	0		1	(4.2)	3	(13.0)
MISALIGNED SLIGHTLY,5TH	MIN	1	(0.4)	3	(1.1)	1	(0.4)	1	(0.4)
		1	(4.2)	3	(13.0)	1	(4.2)	1	(4.3)
RIBS									
KINKED,5TH - UNILATERAL	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
KINKED,6TH - UNILATERAL	MIN	3	(1.1)	0		0		0	
		1	(4.2)	0		0		0	
KINKED,7TH - UNILATERAL	MIN	3	(1.1)	0		0		0	
		1	(4.2)	0		0		0	
KINKED,8TH - UNILATERAL	MIN	2	(0.7)	1	(0.4)	0		0	
		1	(4.2)	1	(4.3)	0		0	
KINKED,9TH - UNILATERAL	MIN	3	(1.1)	0		1	(0.4)	0	
		1	(4.2)	0		1	(4.2)	0	

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		0 ppm		300 ppm		1800 ppm		12000 ppm	
	CLASS	INCIDENCE BY FOETUS/LITTER							
		NO.	%	NO.	%	NO.	%	NO.	%
RIBS									
KINKED,10TH - UNILATERAL	MIN	5	(1.8)	0		0		0	
		3	(12.5)	0		0		0	
KINKED,11TH - UNILATERAL	MIN	5	(1.8)	0		1	(0.4)	0	
		3	(12.5)	0		1	(4.2)	0	
KINKED,12TH - UNILATERAL	MIN	3	(1.1)	0		1	(0.4)	0	
		2	(8.3)	0		1	(4.2)	0	
KINKED,13TH - UNILATERAL	MIN	3	(1.1)	0		0		0	
		2	(8.3)	0		0		0	
THICKENED MID POINT,5TH - UNILATERAL	MIN	4	(1.4)	0		0		0	
		2	(8.3)	0		0		0	
THICKENED MID POINT,6TH - UNILATERAL	MIN	4	(1.4)	0		0		0	
		2	(8.3)	0		0		0	
THICKENED MID POINT,7TH - UNILATERAL	MIN	3	(1.1)	0		1	(0.4)	0	
		1	(4.2)	0		1	(4.2)	0	
THICKENED MID POINT,8TH - UNILATERAL	MIN	3	(1.1)	0		0		0	
		1	(4.2)	0		0		0	
THICKENED MID POINT,9TH - UNILATERAL	MIN	4	(1.4)	0		1	(0.4)	0	
		2	(8.3)	0		1	(4.2)	0	
THICKENED MID POINT,10TH - UNILATERAL	MIN	4	(1.4)	0		1	(0.4)	6	(2.5)
		2	(8.3)	0		1	(4.2)	4	(17.4)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

TABLE 11 (continued)

INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

	CLASS	0 ppm INCIDENCE BY FOETUS/LITTER NO.	%	300 ppm NO.	%	1800 ppm NO.	%	12000 ppm NO.	%
RIBS									
THICKENED MID POINT,11TH - UNILATERAL	MIN	2	(0.7)	0		0		0	
		2	(8.3)	0		0		0	
THICKENED MID POINT,12TH - UNILATERAL	MIN	1	(0.4)	0		0		0	
		1	(4.2)	0		0		0	
EXTRA RIBS									
14TH - UNILATERAL - NORMAL LENGTH	VAR	0		0		1	(0.4)	0	
		0		0		1	(4.2)	0	
14TH - UNILATERAL - SHORT LENGTH	VAR	32	(11.3)	12	(4.6)	31	(11.2)	40	(16.5)
		12	(50.0)	8	(34.8)	12	(50.0)	16	(69.6)
PELVIC GIRDLE									
ASYMMETRIC ALIGNMENT	MIN	0		1	(0.4)	0		0	
		0		1	(4.3)	0		0	
CALCANEUM									
NOT OSSIFIED - UNILATERAL	VAR	108	(38.3)	100	(38.0)	108	(38.8)	121**	(49.8)
		20	(83.3)	18	(78.3)	20	(83.3)	19	(82.6)

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

TABLE 12

INTERGROUP COMPARISON OF MANUS/PES ASSESSMENT

	Dose Level of DEHA (ppm)				Approx 95% Conf Limit
	0	300	1800	12000	
Manus	No. %	No. %	No. %	No. %	
Score 1	0 0.0	0 0.0	0 0.0	0 0.0	
2	172 61.0	171 65.0	195 70.1	140 57.6	
3	103 36.5	92 35.0	83 29.9	102 42.0	
4	7 2.5	0 0.0	0 0.0	1 0.4	
Mean Score	2.40 (2.36)	2.34 (2.34)	2.30 (2.31)	2.44 (2.44)	±0.12 (±0.11)
Pes	No. %	No. %	No. %	No. %	
Score 1	0 0.0	0 0.0	0 0.0	0 0.0	
2	29 10.3	29 11.1	21 7.6	5 2.1	
3	247 87.6	232 88.5	256 92.1	236 97.1	
4	6 2.1	1 0.4	1 0.4	2 0.8	
Mean Score	2.92 (2.90)	2.90 (2.90)	2.92 (2.93)	2.99 (2.99*)	±0.07 (±0.07)
No of litters examined	24 (23)	23 (23)	24 (24)	23 (23)	
No of foetuses examined	282 (269)	263+ (263)+	278 (278)	243 (243)	

Values and comparisons omitting foetuses from Female No 7 are shown in parentheses.
+ Pes scores for 262 foetuses.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX A

ANALYSIS OF DEHA

	% w/w
Purity (as ester) - GLC	99.2
Phthalate (as DOP) - UV spectrophotometer	0.08
Free alcohol	0.02
Water	0.04
Acid value (mg KOH/g)	0.017

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX B

THE CONSTITUENTS OF CT1 DIET

CT1 diet was supplied as a meal in 25kg quantities which were wrapped in 5 ply paper sacks. An analysis of each batch of diet for major constituents and contaminants was supplied by the manufacturer, Special Diets Services Limited. This was checked for acceptability, (based on the best available information at the time) before the batch was used.

The diet and water used were considered not to contain any additional substance in sufficient concentration to have an influence on the outcome of the study.

CT1 is prepared from the following fixed formula:

	% w/w
Cornflour	10.0
Wheat bran	15.0
Wheat	20.0
Maize	10.0
Wheat Feed	20.0
Soya Hypro 50	8.0
Unextracted Yeast	2.5
Denatured Skim Milk Powder*	7.5
White Fish Meal	5.0
PCD Premix	2.0

* Denatured skim milk powder has the following formula:

Skim Milk Powder	72%
White Fish Meal	28%

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX B - continued

THE CONSTITUENTS OF CT1 DIET

When used at 2% inclusion rate (20kg/tonne) PCD premix contributes the following:-

Vitamin A	8.0mIU	Choline	150.0g
Vitamin D_3	1.0mIU	Iron	30.0g
Vitamin E	62.5g	Cobalt	0.4g
Vitamin B_2	8.0g	Manganese	25.0g
Vitamin K M.S.B.	10.0g	Copper	7.0g
Nicotinic Acid	20.0g	Iodine	1.3g
Pantothenic Acid	4.4g	Magnesium	103.0g
Folic Acid	6.0g	Sodium Chloride	5000.0g
Vitamin B_1	2.0g	Phosphorus	1200.0g
Vitamin B_{12}	12.0mg	Calcium	4480.0g

All batches of CT1 diet comply with the following specification with respect to the maximum permitted levels of contaminants.

Contaminant	Maximum permitted level (ppm)	
Selenium	(min)	0.025
Selenium	(max)	0.5
Cadmium		0.8
Mercury		0.2
Arsenic		1.0
Lead		3.0
PCB's	(total)	0.15
DDT's	(total)	0.3
Dieldrin		0.05
Lindane		0.1
Heptachlor		0.05
Malathion		5.0
Nitrite		5.0
Nitrate		150.0
Aflatoxin		0.01

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX C

DIET PREPARATION

The experimenal diets were prepared in 30kg batches from premixes using the quantities of DEHA (adjusted for the 99.2% w/w purity) and size of premix detailed below.

Dietary Conc (ppm)	Amount of DEHA (g)	Premix (kg)	'Bulk' Diet (kg)
0	0	1	29
300	9.07	1	29
1800	54.44	2	28
12000	362.90	4	26

The premixes were made 1kg at a time, using the following procedure. The DEHA was divided into 4 approximately equal quantities for the 12000ppm dose and 2 approximately equal quantities for the 1800ppm dose. A portion of DEHA was added to 500g diet and mixed in a pestle and mortar. A little diet was added to the compound bottle to remove any remaining DEHA and this was added to the premix. The remaining 500g diet was then slowly added and mixed in the pestle and mortar to form a dry premix. This was added to the approriate quantity of 'bulk' diet. The process was repeated as appropriate from the 1800 and 12000ppm diets and the whole diet then mixed thoroughly using a Fielder mixer. A similar process was used for the control diet except that no DEHA was added.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX D

THE DETERMINATION OF DEHA IN DIET

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX D (1)

THE DETERMINATION OF DEHA IN DIET BY SOXHLET EXTRACTION

METHOD SUMMARY

Accurately weighed diet samples were Soxhlet extracted with hexane. The extract solutions were diluted with hexane to give solutions containing nominally 108-120μg/ml DEHA.

These solutions were analysed by capillary gas chromatography with a flame-ionisation detector. The areas of the peaks due to DEHA were used to calculate dietary concentrations.

CHEMICALS

Hexane, HPLC grade - Rathburn Chemicals.

CALIBRATION STANDARDS

Preparation of Stock Solution

DEHA (nominally 150mg), CTL reference YO2259/003/001, purity 99.2% w/w was accurately weighed into a 50ml standard flask, dissolved in hexane and diluted to 50ml (nominally 3mg/ml).

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

APPENDIX D (1) - continued

THE DETERMINATION OF DEHA IN DIET
BY SOXHLET EXTRACTION

Preparation of Working Standard Solutions

Portions of the stock solution (1.0, 2.0, 3.0 and 4.0ml) were each diluted to 50ml with hexane to give solutions containing 60, 120, 180 and 240µg/ml DEHA respectively.

PROCEDURE

(a) Preparation of Recovery Diet Samples

300ppm: Aliquots (1.0ml) of the DEHA stock solution were added by pipette to each of three 10g portions of control diet contained in 100ml beakers. The diets were stirred with glass rods, left for at least 2 hours, then transferred with a small volume of hexane to Soxhlet extraction thimbles (22 x 80mm).

1800ppm: Accurately weighed portions (nominally 18mg) of DEHA (YO2259/003/001) were weighed into three 100ml beakers. Control diet (10g) was added, the contents stirred with a glass rod and transferred to extraction thimbles with a small volume of hexane.

12000ppm: An accurately weighed portion (nominally 1200mg) DEHA (YO2259/003/001) was weighed into a 100ml beaker. A 100g portion of control diet was weighed separately. DEHA was transferred with added portions of control diet to a pestle and mortar to effect a quantitative transfer. The mixture was ground for approximately 5 minutes to obtain a fine intimate mix and finally mixed on a Stuart Flask Rotator for 30 minutes at Speed 6 in a 500ml stoppered conical flask. Three 10g portions were weighed into extraction thimbles.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

APPENDIX D (1) - continued

THE DETERMINATION OF DEHA IN DIET
BY SOXHLET EXTRACTION

(b) Extraction

Duplicate 10g portions of diet were weighed into Soxhlet extraction thimbles (22 x 80mm) and these transferred to Soxhlet extractors. Hexane (100ml) was added to 250ml round-bottomed flasks and the necessary components assembled to allow Soxhlet extraction to take place. Samples were extracted for 3 hours and the extract solutions evaporated to approximately 10ml by rotary evaporation under reduced pressure. Extract solutions were transferred with hexane to appropriate standard volumetric flasks and diluted to volume with hexane. Further dilutions in hexane were carried out if required to give solutions containing nominally 120μg/ml (300, 12000ppm) or 108μg/ml DEHA (1800ppm). Control diet extracts were treated in the same manner as the 300ppm samples.

(c) Gas-Liquid Chromatography

Gas chromatograph	: Carlo Erba HRGC 5300 Mega Series
Column	: 007 Series bonded phase fused silica capillary column, 15m x 0.53mm id, 1.0μm film thickness, methyl 50% phenyl silicone (Quadrex Corporation)
Column oven temperature	: 210°C, programmed to 240°C at 12°C/min, held for 4 min. Alternatively, 200°C held for 1 min, programmed at 10°C/min to 240°C, held for 2 or 3 min
Detector	: Flame-ionisation

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX D (1) - continued

THE DETERMINATION OF DEHA IN DIET BY SOXHLET EXTRACTION

Detector oven temperature	: 300°C
Carrier gas	: Helium at 0.85 or 1kg/cm^2
Make up gas	: Argon/methane (95:5 v/v) at 0.7kg/cm^2
Detector gases	: Air (1.5kg/cm^2), hydrogen (0.8kg/cm^2)
Injection	: 1μl, HOT injector (Carlo Erba), on-column cooling for 30 seconds
Data Handling	: Trilab 2000 (Trivector Scientific)

Sample solutions and the calibration standard solutions were transferred to vials. These were arranged on the sample carousel so that all the calibration standard solutions were injected at the start of a run and the 120μg/ml standard interspersed at regular intervals during the run. The mean peak area value was calculated for each sample solution. A calibration graph was constructed by input of mean peak area values for standard solutions to a statistics computer programme to produce a linear regression plot. The mean peak area values obtained for sample extract solutions were then entered and a concentration value (Cμg/ml) obtained. Alternatively, results were calculated against a mean value for the nominal standard of 120μg/ml.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

APPENDIX D (1) - continued

THE DETERMINATION OF DEHA IN DIET
BY SOXHLET EXTRACTION

CALCULATION OF RESULTS

(a) Recovery Determination

The following equation was used to calculate the % recovery of DEHA in diet:

$$\% \text{ recovery} = \frac{C_S \times D_F \times 100}{W \times T}$$

C_S = concentration of DEHA in analysed recovery samples (μg/ml)
D_F = dilution factor (ml)
W = sample weight (10g)
T = target level for recovery samples (ppm w/w)

(b) Calculation of the Dietary Levels of DEHA

The following equation was used to calculate the level of incorporation of DEHA in diet:

$$\text{ppm (w/w) DEHA} = \frac{C_S \times D_F \times P}{10 \times R}$$

C_S = concentration of DEHA in analysed samples (μg/ml)
D_F = dilution factor (ml)
P = purity of reference material (99.2% w/w)
R = % recovery

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX D (1) - continued

THE DETERMINATION OF DEHA IN DIET BY SOXHLET EXTRACTION

LIMIT OF DETERMINATION

The limit of determination was set at 10ppm for DEHA in diet.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX D (2)

THE DETERMINATION OF DEHA IN DIET BY VORTEX EXTRACTION

METHOD SUMMARY

Accurately weighed diet samples were extracted with hexane on a Vortex mixer. Extract solutions were diluted if required to give solutions containing nominally 144-150μg/ml DEHA.

These solutions were analysed by capillary gas chromatography with a flame-ionisation detector. The areas of the peaks due to DEHA were used to calculate the dietary concentration.

CHEMICALS

Hexane, HPLC grade - Rathburn Chemicals.

CALIBRATION STANDARDS

Preparation of Stock Solution

DEHA (nominally 250mg), CTL reference Y02259/003/001, purity 99.2% w/w, was accurately weighed into a 50ml standard flask, the test substance dissolved in hexane and diluted to 50ml (nominally 5mg/ml).

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX D (2) - continued

THE DETERMINATION OF DEHA IN DIET BY VORTEX EXTRACTION

Preparation of Working Standard Solutions

Portions of the stock solution (2.0, 3.0, 4.0 and 5.0ml) were each diluted to 100ml with hexane to give solutions containing nominally 100, 150, 200 and 250μg/ml DEHA respectively.

PROCEDURE

(a) Preparation of Recovery Diet Samples

Typically these were prepared as follows:-

300ppm

DEHA (nominally 75mg), CTL reference Y02259/003/001 was dissolved in hexane and diluted to 25ml in a standard flask (3mg/ml). Portions (200μl) of this solution were added to each of three 2g amounts of control diet. After mixing with a glass pasteur pipette the diets were allowed to stand at room temperature overnight.

1800ppm

DEHA (nominally 450mg), CTL reference Y02259/003/001 was dissolved in hexane and diluted to 25ml in a standard flask (18mg/ml). Portions (200μl) were added to triplicate 2g amounts of control diet and treated as described above.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

APPENDIX D (2) - continued

THE DETERMINATION OF DEHA IN DIET
BY VORTEX EXTRACTION

12000ppm

DEHA (nominally 24mg), CTL reference Y02259/003/001 was weighed into glass tubes. Control diet (2g) was added and the tube contents mixed with a glass pasteur pipette.

(b) Extraction

Approximately 10g portions of each test diet was ground using a pestle and mortar. Duplicate 2g portions of the ground sample were accurately weighed into screw-capped glass tubes. To control and 300ppm diet, 4.0ml hexane was added. To diets at other levels, 5ml hexane was added. Samples were vortex mixed (Gallenkamp Spin Mix) for 60 seconds, then centrifuged for 10 min at 1500rpm (MSE Mistral 4L). Extract solutions were transferred to vials and diluted with hexane if required to give solutions containing nominally 144-150μg/ml DEHA.

(c) Gas-liquid Chromatography

Gas Chromatograph	: Carlo Erba HRGC 5300 Mega Series or a Pye Unicam 204.
Column	: 007 series bonded phase fused silica capillary column, 15m x 0.53mm id, 1.0μm film thickness, methyl 50% phenyl silicone (Quadrex Corporation).
Column Oven Temperature:	Typically 210°C, hold for 1 min, programmed at 12°C/min to 240°C, hold for 4 min. Minor variations on these conditions were used on occasions.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

APPENDIX D (2) - continued

THE DETERMINATION OF DEHA IN DIET
BY VORTEX EXTRACTION

Detector	: Flame-ionisation.
Detector Oven Temperature	: 300°C
Carrier Gas	: Helium, 1kg/cm^2
Make Up Gas	: Argon/methane, 95:5v/v, 0.7kg/cm^2
Detector Gases	: Hydrogen 0.8kg/cm^2, air 1.5kg/cm^2
Injection	: 1μl, HOT injector (Carlo Erba) on-column cooling for 30 seconds.
Data Handling	: Trilab 2000 (Trivector Scientific).

Alternative conditions employed were as follows:-

Gas Chromatograph	: Pye Unicam 204
Column	: BP1, 15m x 0.53mm id fused silica
Column Temperature	: 210°C, hold for 1 min, programmed at 12°C/min to 250°C, hold for 2 min.
Carrier Gas	: Nitrogen, 7 lb/in^2
Injection	: 2μl, manual

Sample solutions and the calibration standard solutions were transferred to vials. These were arranged on the sample carousel so that all the calibration standard solutions were injected at the start of a run and the 150μg/ml standard interspersed at regular intervals during the run. The mean peak area value was calculated for each sample solution. A calibration graph was constructed by input of mean peak area values for standard solutions to a statistics computer programme to produce a linear regression plot. The mean peak area values obtained for sample extract solutions were then entered and a concentration value (Cμg/ml) obtained. Alternatively concentrations were calculated by direct proportion to a bracketed mean peak area value obtained for the 150μg/ml standard.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

APPENDIX D (2) - continued

THE DETERMINATION OF DEHA IN DIET
BY VORTEX EXTRACTION

CALCULATION OF RESULTS

(a) Recovery Determination

The following equation was used to calculate the % recovery of DEHA in diet:-

$$\% \text{ recovery} = \frac{C_S \times D_F \times 100}{W \times T}$$

C_S = concentration of DEHA in analysed recovery samples (μg/ml)
D_F = dilution factor (ml)
W = sample weight (2g)
T = target level for recovery samples (ppm w/w)

(b) Calculation of the Dietary Levels of DEHA

The following equation was used to calculate the level of incorporation of DEHA in diet:-

$$\text{ppm (w/w) DEHA} = \frac{C_S \times D_F \times P}{2 \times R}$$

C_S = concentration of DEHA in analysed samples (μg/ml)
D_F = dilution factor (ml)
P = purity of reference material (99.2% w/w)
R = % recovery

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX D (2) - continued

THE DETERMINATION OF DEHA IN DIET BY VORTEX EXTRACTION

LIMIT OF DETERMINATION

The limit of determination was set at 10ppm for DEHA in diet.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX E

CHEMICAL STABILITY OF DEHA IN DIET (DATA PRODUCED ON A CONCURRENT STUDY)

Preparation Date	Nominal Concn (ppm w/w)	Extraction Date	Analysis Interval (days)	Analysed Concn (ppm w/w)	Mean Concn (ppm w/w)	% of Initial Value
5 Aug 87	300	6 Aug 87†	0	348, 315	332	100.0
		24 Aug 87	18	271, 329	300	90.4
		2 Sep 87	27	325, 292	309	93.1
	12000	6 Aug 87†	0	12450, 12770	12610	100.0
		24 Aug 87	18	12080, 11720	11900	94.4
		2 Sep 87	27	11990, 11620	11810	93.7
23 Aug 87	300	24 Aug 87	0	289, 292	291	100.0
		2 Sep 87	9	309, 262	286	98.3
		9 Sep 87	16	226, 285	256	88.0
		23 Sep 87	30	277, 255	266	91.4
	12000	24 Aug 87	0	11650, 12300	11980	100.0
		2 Sep 87	9	12180, 12160	12170	101.6
		9 Sep 87	16	12090, 11740	11920	99.5
		23 Sep 87	30	11710, 11310	11510	96.1

† These analyses were carried out using a rapid vortex extraction on 2g samples. Subsequent work showed that whilst this technique appears satisfactory with freshly prepared diet, low results were obtained on aged diet. Therefore all subsequent analysis of samples for stability was performed by the method described in Appendix D (1).

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX E - continued

CHEMICAL STABILITY OF DEHA IN DIET (DATA PRODUCED ON A CONCURRENT STUDY)

Preparation Date	Nominal Concn (ppm w/w)	Extraction Date	Analysis Interval (days)	Analysed Concn (ppm w/w)	Mean Concn (ppm w/w)	% of Initial Value
31 Oct 87	300	3 Nov 87	0	286, 288	287	100.0
		19 Nov 87	16	285, 293	289	100.7
		7 Dec 87	34	290, 285	288	100.3
	12000	3 Nov 87	0	11860, 12330	12100	100.0
		19 Nov 87	16	11680, 12070	11880	98.2
		7 Dec 87	34	11840, 12180	12010	99.3

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX F

ARRANGEMENT OF ANIMALS AND EXPERIMENTAL GROUPS ON THE RACKS

	RACK 1				RACK 2				RACK 3				RACK 4				RACK 5			
Replicate No.	1	1	1	1	6	6	6	6	11	11	11	11	16	16	16	16	21	21	21	21
Animal No.	25	49	73	1	30	6	78	54	59	11	35	83	64	40	16	88	21	93	69	45
Group No.	2	3	4	1	2	1	4	3	3	1	2	4	3	2	1	4	1	4	3	2
Replicate No.	2	2	2	2	7	7	7	7	12	12	12	12	17	17	17	17	22	22	22	22
Animal No.	74	2	26	50	79	55	31	7	12	36	84	60	65	89	41	17	94	22	46	70
Group No.	4	1	2	3	4	3	2	1	1	2	4	3	3	4	2	1	4	1	2	3
Replicate No.	3	3	3	3	8	8	8	8	13	13	13	13	18	18	18	18	23	23	23	23
Animal No.	3	27	51	75	8	80	56	32	85	13	37	61	90	42	18	66	47	71	95	23
Group No.	1	2	3	4	1	4	3	2	4	1	2	3	4	2	1	3	2	3	4	1
Replicate No.	4	4	4	4	9	9	9	9	14	14	14	14	19	19	19	19	24	24	24	24
Animal No.	52	76	4	28	33	81	57	9	38	86	62	14	19	67	91	43	72	48	24	96
Group No.	3	4	1	2	2	4	3	1	2	4	3	1	1	3	4	2	3	2	1	4
Replicate No.	5	5	5	5	10	10	10	10	15	15	15	15	20	20	20	20				
Animal No.	53	29	5	77	82	58	10	34	15	63	87	39	44	20	68	92				
Group No.	3	2	1	4	4	3	1	2	1	3	4	2	2	1	3	4				

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY STUDY IN THE RAT

APPENDIX G

SCALE FOR ASSESSMENT OF SKELETAL OSSIFICATION OF THE MANUS AND PES

Scale

1.(good) Metacarpals/metatarsals and first and third row of phalanges fully ossified (or one phalanx partially ossified).

2. Metacarpals/metatarsals fully ossified. First or third row of phalanges ossified, although an occasional phalanx (approximately up to four) may be partially ossified.

3. Metacarpals/metatarsals fully or occasionally partially ossified. First row phalanges either partially or not ossified together with third row of phalanges either partially or fully ossified.

4.(poor) Metacarpals/metatarsals - some either partially or not ossified plus first row of phalanges usually not ossified and third row of phalanges partially ossified.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

APPENDIX H

PERCENTAGES OF PRE- AND POST-IMPLANTATION LOSSES
IN CONTROL GROUPS IN FIVE RECENT STUDIES

Date	% Pre-implantation loss				% Post-implantation loss			
	Group Number				Group Number			
	1	2	3	4	1	2	3	4
May 1985	8				6			
July 1985	5				6			
Apr 1986	17				5			
June 1986	10				7			
Nov 1986	12				4			
Present Study:								
Sept 1987	14	12	12	19	4	3	4	6

Values are presented for all groups for the present study. Only control group values (group 1) are presented for the other 5 studies.

DI(2-ETHYLHEXYL)ADIPATE: TERATOGENICITY
STUDY IN THE RAT

CIRCULATION

Internal

1 Report Centre Reference Copy
2 Report Centre - Spare
3 Dr I F H Purchase)
Dr S E Jaggers)
Dr R S Morrod)
4 Dr G T Steel
5 Mrs D L Kinsey
6 Mr P B Banham
7 Mr M Greenwood
8 Mr M C E Hodge
9 Dr G A Wickramaratne/Dr G H Pigott

External

10 Dr B Berndtsson, Neste OXO AB, Sweden
11 Dr F Carpanini, BP International Ltd, England
12 Dr J Jackson, Monsanto Europe, Belgium
13 Dr R Jackh, BASF Toxicology, Federal Republic Germany
14 Dr R J Millischer, Atochem, France
15 Dr J Rudolph, Huls, Federal Republic Germany
16 Dr C Cella, EVC, Belgium
17 Mr N Sarginson, Exxon Chemicals, Belgium
18 Dr D F Cadogan, ICI Chemicals and Polymers, England
19 Dr C Schneider, BASF, Federal Republic Germany
20 Dr W Pump, Bayer AG, Federal Republic Germany
21 Dr M Wooder, Shell International, Belgium
22 Dr D Starck, Hoechst AG, Federal Republic Germany
23 Dr D M Pugh, BP Chemicals, England
24 Mr C R Perry, Monsanto, Belgium
25 Dr A Seys, CEFIC, Belgium

CH/TP11